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Calcification by *Candida albicans*

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Candida albicans was grown in a chemically defined medium in which certain microorganisms are known to calcify. The fungus developed calcium phosphate deposits with the same X-ray diffraction maxima as biological apatite.

This investigation examined *Candida albicans* for calcification. It is part of a program designed to develop a microbiological model for vertebrate calcifications.

Most microbiological calcification studies have been with the actinomycete, *Bacterionema matruchotii* (1). The organism forms intracellular calcium phosphate deposits during growth in a chemically defined medium (2). The X-ray diffraction maxima of the mineral are the same as biological apatite. Two recent reports (3, 4) have shown that apatite formation by bacteria in pure culture is not restricted to *B. matruchotii*. If the phenomenon were also to occur with a eukaryotic microorganism, a relatively broad spectrum of microbiological calcification under readily controlled conditions would be established.

C. albicans ATCC 10231 was grown at 37 C in 250-ml volumes of the chemically defined medium (2) known to provide for growth and calcification of a variety of microorganisms. Beginning at 7 days, cultures were harvested daily for 4 days by centrifugation at 34,000 × *g* for 5 min at 5 C and washed with water. A small portion of each yield was stored at −15 C. The remainder was dried at 45 C, radiofrequency ashed at 100 C for 2 h, and analyzed by X-ray diffraction. After the 10-day sample had been diffracted, that part of the sample held at −15 C was processed for and examined, unstained, by electron microscopy. Techniques and instrumentation for both X-ray diffraction and electron microscopy have been reported previously (2).

C. albicans grew well in the synthetic medium. Blastospores were the predominant form. During incubations, the pH of the medium ranged from 7.20 to 7.30.

Apatite was detected by X-ray diffraction of the 8-, 9-, and 10-day samples. A representative pattern is illustrated in Fig. 1. For comparison, a diffraction pattern obtained with powdered human dentin is included.

Electron microscopy confirmed the X-ray finding. Needle-like apatite crystals were found in the cytoplasm, in cell walls, and projecting outward from the wall surface (Fig. 2A and B).

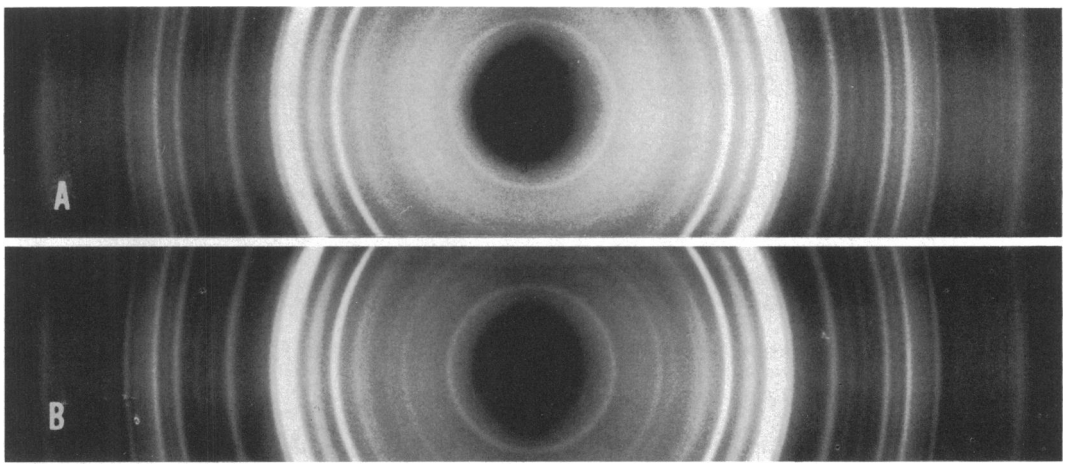

FIG. 1. *Positive prints of X-ray diffraction patterns. (A) Radio-frequency ashed residue of calcified C. albicans; and (B) powdered human dentin.*

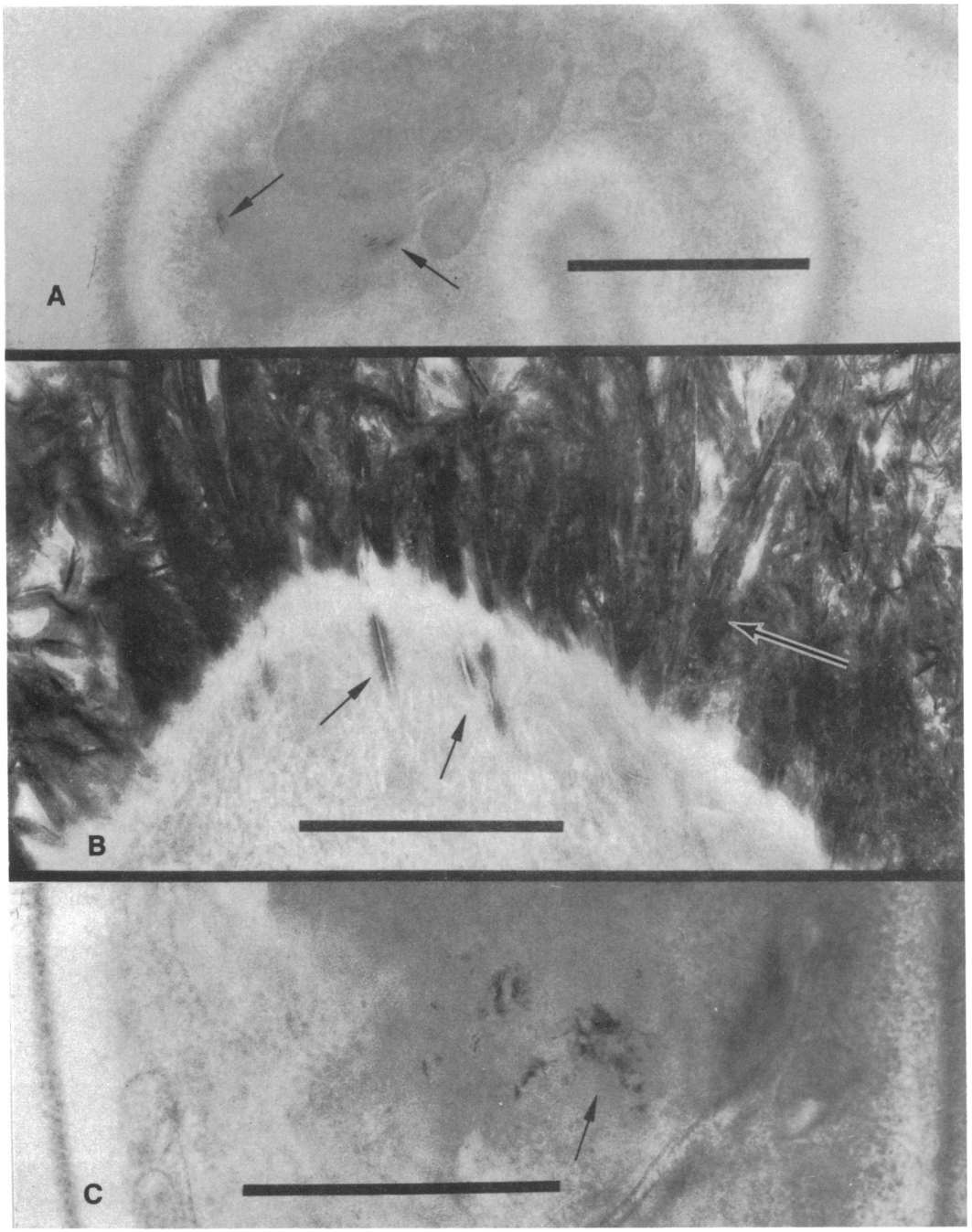

FIG. 2. *Electron micrographs of calcified C. albicans. (A) Apatite crystals (arrow) in cytoplasm; (B) apatite crystals (arrow) in cell wall and extending (arrow) from wall surface; and (C) nonorganized, intracytoplasmic mineral (arrow). Bar represents 0.5 μm.*

There was also intracytoplasmic electron-opaque material that appeared to lack organization (Fig. 2C).

C. albicans formed biological apatite during cultivation in a medium developed originally for calcification by *B. matruchotii*. As with all prior in vitro microbiological calcifications, prolonged incubation was required. Whether this is

related to slow synthesis of an essential, progressive inactivation of an inhibitor or a necessary accumulation of dead cells is not known. The only certainty is that apatite formation was cell dependent. With the medium between pH 7.20 and 7.30, there would be no spontaneous mineral precipitation. Salts remain solubilized in uninoculated medium at pH 7.45 indefinitely.

Apatite crystals were found in small intracytoplasmic clusters and as linear arrangements in cell walls with crystal masses extending peripherally from the wall. The nonstructured, intracytoplasmic electron-opaque material was probably amorphous mineral. Additional interpretation of these findings is not warranted since the cells were harvested and washed by using high-speed centrifugation before fixation. This was done because validity of calcification as determined by X-ray diffraction is more important in an initial study than are the ultrastructural details. Therefore, assigning significance to a mineral arrangement or a mineral-cell relationship would be specious.

Future *C. albicans* calcification studies might well emphasize ultrastructure. The relatively high degree of differentiation in the fungus should provide information not obtainable with bacteria. Of special interest would be to localize calcification in relation to a subcellular component. Of equal significance would be to determine whether the first mineral formed is crystalline or amorphous.

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LITERATURE CITED

1. Ennever, J., and I. Takazoe. 1973. Bacterial calcification, p. 629-648. *In* I. Zipkin (ed.), Biological mineralization. Wiley Interscience, New York.
2. Ennever, J., J. J. Vogel, and J. L. Streckfuss. 1971. Synthetic medium for calcification of *Bacterionema matruchotii*. J. Dent. Res. **50**:1327-1330.
3. Ennever, J., J. J. Vogel, and J. L. Streckfuss. 1974. Calcification by *Escherichia coli*. J. Bacteriol. **119**:1061-1062.
4. Streckfuss, J. L., W. N. Smith, L. R. Brown, and M. M. Campbell. 1974. Calcification of selected strains of *Streptococcus mutans* and *Streptococcus sanguis*. J. Bacteriol. **120**:502-506.